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# Antifungal activity of medicinal plant extracts for potential management of *Fusarium* pathogens

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**ABSTRACT**

Yield losses due to fungal attacks, post-harvest losses and food spoilage, amongst others present a challenge to food security. There is an ongoing need and search for accessible, affordable and environmentally-friendly alternatives to the use of synthetic pesticides in food production. The aim of this study was to search for affordable potent plant extracts that can be used in smallholder farming system to manage *Fusarium* related diseases. Extracts from 11 medicinal plant species previously screened against human and animal pathogens in the literature were selected and investigated for their *in vitro* antifungal activity against five economically important phytopathogenic *Fusarium* species. Dried leaf powders were extracted with solvents of different polarity and evaluated for antifungal activity using a microdilution method. At least, one of the solvent extracts obtained from a minimum of three plant species demonstrated very strong activity with minimum inhibitory concentration (MIC) less than 0.1 mg/ml against *F. equisite*, *F. oxysporum*, *F. semitectum*, *F. chlamydosporum* and *F. subglutinans*. Acetone and ethyl acetate solvent extracts were found in most cases to exhibit stronger antifungal activity compared to water and petroleum ether extracts. However, water extract of *Combretum molle* was particularly noteworthy as it demonstrated antifungal activity against the tested five *Fusarium* species. The use of medicinal plant extracts as an antifungal agent presented a cheap, accessible and sustainable source of eco-friendly pesticides useful for crop protection in organic cultivation and small-holder farming.

**Key words :** *Fusarium*, organic farming, plant diseases, plant extracts, synthetic fungicides

## INTRODUCTION

Smallholder farming remains a source of food and income generation for many households in rural communities. Maize, cowpea, sweet potatoes, soybeans and tomatoes are among major crops cultivated by smallholder farmers. These crops provide carbohydrates, proteins and vitamins required for human well-being. A successful production of these crops can be limited by a number of plant diseases such as vascular wilting, head blight, damping-off, ear and root rots caused by *Fusarium* pathogens. Plant disease is problematic in farming as it negatively impacts on and results in poor crop quality or commodities. It may result in massive yield losses both in the field and during storage (Oren *et al.*, 2003).

*Fusarium* species are soil-borne fungal pathogens; however, when conditions are favourable they can cause spoilage of crops, kernels, grains and fruits during post-harvest processes and storage (Smith, 1986). Some *Fusarium* species are capable of producing mycotoxins and allergens which contaminate food and invariably pose health risks to consumers of contaminated products (Gelderblom *et al.*, 1988; Marasas, 1995; Shephard *et al.*, 1996; Fandohan *et al.*, 2005; Omidpanah *et al.*, 2015). Synthetic fungicides have been beneficial to the agricultural sector for many decades and their importance in the reduction of plant diseases cannot be overlooked. Although they remain an important part of efficient plant disease management practice, synthetic fungicides are not easily accessible to, or affordable for smallholder or

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subsistence farmers. This kind of farming in particular is more often practised in poor rural communities (Stephens *et al.*, 1989; Thembo *et al.*, 2010).

The use of synthetic fungicides in agricultural production is being increasingly discouraged due to their harmful effects on non-target organisms in conjunction with the contamination of aquatic systems and the development of pathogen-resistant strains (Lalitha *et al.*, 2010; Thippeswamy *et al.*, 2011; Adepoju *et al.*, 2014; Silva *et al.*, 2014; Dube and Maleka, 2017). The problems associated with food security and the applications of synthetic fungicides necessitate the need to search for affordable and environmental-friendly fungicides. Therefore, this study was aimed at investigating the *in vitro* antifungal activity of selected medicinal plants for potential treatment of plant diseases caused by *Fusarium* pathogens. The medicinal plant species used in this study were selected from available literature based on their documented strong antimicrobial activity when tested against human and/or animal fungal pathogens.

## MATERIALS AND METHODS

### Collection of Plant Materials

The leaves of *Lantana camara*, *Combretum erythrophyllum*, *Solanum mauritianum*, *Melia azedarach*, *Combretum molle, Olea europea* and *Quercus acutissima* were collected from the Agricultural Research Council, Roodeplaat Campus, South Africa. The leaves of other plants, which are *Senna didymobotrya*, *Harpephyllum caffrum*, *Withania somnifera* and *Vangueria infausta*, were collected from Capricorn district in Limpopo Province of South Africa. Voucher specimens were prepared for all these species and deposited at the University of Limpopo herbarium, South Africa.

### Preparation of Plant Extracts

The leaves were shade-dried at room temperature (25±2°C) and grinded into fine powder using Fritsch Pulverisette 14 milling machine with 0.5 mm sieve ring. The plant materials were subsequently extracted non-sequentially with water, petroleum ether, ethyl acetate and acetone by sonicating in an ultrasonic bath for 1 h. The extracts were then filtered through Whatman No.1 filter paper and the organic filtrate was dried in a fume hood. The water filtrate was lyophilized using a freeze dryer (Sentry 2.0 VirTis SP, United Scientific). The extractant yield was calculated and expressed in milligram/gram dry plant material. The dried organic extracts (petroleum ether, ethyl acetate and acetone) were re-constituted at a 10 mg/ml working concentration.

### Preparation of Fungal Pathogens

*Fusarium oxysporum* (PPRI 10175), *F. semitectum* (PPRI 6739), *F. chlamydosporum* (PPRI 5116), *F. equiseti* (PPRI 19029) and *F. subglutinans* (PPRI 6740) were obtained from Plant Health and Protection Campus of the Agricultural Research Council, South Africa. The pathogens were cultured on potato dextrose agar and incubated at 27°C for four days. Afterwards, fungal suspension was scrapped off, inoculated into potato dextrose broth and incubated for three days. The spores were collected by straining the cultured broth through sterile cheesecloth; thereafter the concentration was determined using a haemocytometer and microscope (Aberkane *et al.*, 2002). The final spore concentration was adjusted to $1.0 \times 10^6$ fungal spores/ml prior to use in the antifungal assay (Mahlo *et al.*, 2010).

### *In vitro* Antifungal Activity of Plant Extracts

A modified 96-well micro-plate dilution assay (Masoko *et al.*, 2005) was used to determine the antifungal activity of the extracts. The assay was carried out by adding 100 µl plant extract into the first well of the plate and then serially diluted two-fold with sterile potato dextrose broth. One hundred microliters of fungal culture adjusted to $1.0 \times 10^6$ spores/ml were added to each well. Amphotericin B (purchased from Phytotek Lab, South Africa) was used as a positive control, while 50% acetone, potato dextrose broth and sterile water were used as negative controls. The plate was covered and incubated at 27°C for two days. After the incubation period, 50 µl of 0.5 mg/ml *p*-iodonitrotetrazolium chloride (INT) was added to each well and the plate was incubated further overnight. The minimum

inhibitory concentration (MIC), which was the lowest concentration of plant extract that inhibited the growth of fungal pathogen, was recorded for each extract. Minimum inhibitory dilution (MID), indicating how much the extract derived from 1 g plant sample can be diluted to still inhibit fungal growth, was also recorded (Eloff, 2004; Amoo *et al.*, 2012).

## RESULTS AND DISCUSSION

Subsistence or smallholder farming has been a source of food and income generation for households especially in many rural communities. However, yield losses due to *Fusarium* pathogens, amongst others, have been a problem through the years. Application of synthetic fungicides, which is generally considered as not eco-friendly, as control measures in this kind of farming system proved to be challenging as most households could not afford the chemicals. The increasing global preference and demand for organic production in organic farming system required crop protection measures employing organic practices. In this study, medicinal plant extracts were evaluated *in vitro* for possible control of *Fusarium* pathogens such as *F. equisite*, *F. oxysporum*, *F. semitectum*, *F. chlamydosporum* and *F. subglutinans*. A total of 44 extracts from 11 medicinal plant species were investigated for antifungal activity.

As shown in Fig. 1, extraction with water as a solvent gave more yield compared to the use of other solvents. The more polar the solvent used for extraction, the higher the yield recorded. For example, the yield of water extract obtained from *Senna didymobotrya* was 11.5-fold of what was recorded with its petroleum ether extract (Fig. 1). Of the four solvents used, extraction with water gave the highest yield of crude extract. It was demonstrated in several studies that extraction of plant material with polar solvents resulted in greater yield compared to non-polar solvents (Eloff, 1998; Lekganyane *et al.*, 2012; Masoko and Makgapeetja, 2015).

Table 1 shows the antifungal activity based on minimum inhibitory concentrations of different medicinal plant species extracts evaluated against the five *Fusarium* species. At least, one of the solvent extracts obtained from three, five, four, six and eight plant species demonstrated very strong activity with

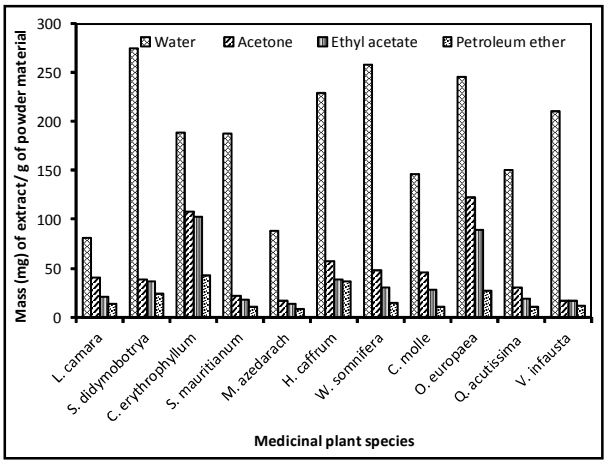


Fig 1. Mass of different crude extracts obtained per gram of plant material.

minimum inhibitory concentration (MIC) less than 0.1 mg/ml against *F. equisite*, *F. oxysporum*, *F. semitectum*, *F. chlamydosporum*, and *F. subglutinans*, respectively. Of the 44 plant extracts tested against the five pathogens; 18, 17, 7 and 6 extracts obtained from acetone, ethyl acetate, petroleum ether and water, respectively, showed very strong activity with MIC <0.1 mg/ml. In general, acetone and ethyl acetate solvent extracts were found in most cases to exhibit stronger antifungal activity compared to water and petroleum ether extracts.

Although crude extract obtained through water extraction was the highest in all the investigated species, its antifungal activity on average was not as strong as that of the other solvents used. This observation correlated with the findings from several authors who reported that aqueous extract generally exhibited little or no antimicrobial activity compared to non-polar extract (Parekh and Chanda, 2007; Van Vuuren and Naidoo, 2010; Kitonde *et al.*, 2014). This might be due to lower solubility of antifungal compounds in polar solvent as compared to non-polar solvents in such medicinal plant species (Bhattacharjee *et al.*, 2011). Nevertheless, *Senna didymobotrya, Solanum mauritianum* and *Melia azedarach* water extracts showed very strong activity against *F. subglutinans,* while similar preparations from *Combretum molle, Olea europaea* and *Vangueria infausta* were strongly active against *F. chlamydosporum*. Water extract of *Combretum molle* was particularly noteworthy as it demonstrated clear antifungal activity (MIC≤2.5 mg/ml) against the tested five

**Table 1.** Minimum inhibitory concentration (MIC) values of medicinal plant species tested against five pathogenic *Fusarium* species

| Plant species | Family | Voucher number | Extract | MIC values (mg/ml) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *F. equisite* | *F. oxysporum* | *F. semitectum* | *F. chlamydosporum* | *F. subglutinans* |
| *Lantana camara* L. | Verbenaceae | UNIN 121003 | WA | 0.63 | 2.5 | >2.5 | >2.5 | 0.16 |
| | | | PE | 0.63 | 1.25 | 1.25 | 0.16 | **0.04** |
| | | | EA | 0.31 | 0.31 | **0.08** | 2.5 | **0.04** |
| | | | AC | 0.16 | 0.63 | **0.04** | 2.5 | **0.04** |
| *Senna didymobotrya* (Fresen.) H. S. Irwin & Barneby | Leguminosae | UNIN 121004 | WA | 0.16 | 1.25 | >2.5 | >2.5 | **0.04** |
| | | | PE | 0.16 | 0.31 | 2.5 | 0.63 | **0.08** |
| | | | EA | 0.31 | 0.16 | 1.25 | 1.25 | **0.04** |
| | | | AC | 0.31 | 0.16 | 1.25 | >2.5 | **0.08** |
| *Combretum erythrophyllum* (Burch.) Sond. | Combretaceae | UNIN 121005 | WA | 0.63 | >2.5 | 1.25 | 0.63 | 1.25 |
| | | | PE | 0.31 | 0.04 | 0.63 | 0.63 | **0.04** |
| | | | EA | 0.16 | 0.16 | 0.31 | **0.04** | **0.04** |
| | | | AC | **0.08** | **0.04** | 0.31 | **0.04** | **0.08** |
| *Solanum mauritianum* Scop. | Solanaceae | UNIN 121006 | WA | 0.16 | 2.5 | >2.5 | >2.5 | **0.08** |
| | | | PE | 0.31 | 0.31 | 0.63 | 0.31 | **0.16** |
| | | | EA | **0.08** | 0.08 | 1.25 | 0.31 | **0.04** |
| | | | AC | 0.31 | **0.04** | 1.25 | 0.31 | **0.04** |
| *Melia azedarach* L. | Meliaceae | UNIN 121007 | WA | 0.63 | 2.5 | >2.5 | >2.5 | **0.08** |
| | | | PE | 0.31 | 0.16 | 2.5 | 0.31 | 0.16 |
| | | | EA | 0.16 | **0.08** | 0.31 | 0.63 | 0.16 |
| | | | AC | 0.16 | 0.16 | 0.63 | **0.04** | **0.08** |
| *Harpephyllum caffrum* Bernh. | Anacardiaceae | UNIN 121002 | WA | 0.31 | >2.5 | >2.5 | >2.5 | 1.25 |
| | | | PE | 0.31 | 0.31 | >2.5 | 0.16 | 0.31 |
| | | | EA | 0.16 | 0.16 | >2.5 | 1.25 | **0.08** |
| | | | AC | 0.16 | 0.31 | 1.25 | 0.16 | 0.31 |
| *Withania somnifera* (L.) Dunal | Solanaceae | UNIN 121010 | WA | 1.25 | 1.25 | >2.5 | >2.5 | 0.16 |
| | | | PE | 0.63 | 0.16 | 0.63 | 0.63 | 0.08 |
| | | | EA | 0.16 | **0.08** | **0.04** | 1.25 | 0.63 |
| | | | AC | 0.31 | **0.08** | **0.08** | 0.63 | 0.31 |
| *Combretum molle* R. Br. ex G. Don | Combretaceae | UNIN 121013 | WA | 0.63 | 1.25 | 2.5 | **0.04** | 1.25 |
| | | | PE | 0.31 | 0.31 | 0.63 | 0.63 | 0.63 |
| | | | EA | 0.16 | 0.16 | **0.04** | **0.04** | 0.16 |
| | | | AC | 0.31 | 0.16 | **0.08** | **0.04** | 0.63 |
| *Olea europaea* L. | Oleaceae | UNIN 121014 | WA | 0.63 | 1.25 | >2.5 | **0.04** | 0.31 |
| | | | PE | 1.25 | 0.63 | 2.5 | **0.04** | 0.31 |
| | | | EA | 0.31 | 0.31 | 1.25 | 1.25 | 0.31 |
| | | | AC | 0.31 | 0.31 | 1.25 | >2.5 | 0.31 |
| *Quercus acutissima* Carruth. | Fagaceae | UNIN 121015 | WA | 1.25 | >2.5 | >2.5 | 0.16 | 1.25 |
| | | | PE | 0.31 | 0.16 | 0.63 | **0.04** | 0.16 |
| | | | EA | 0.16 | **0.08** | 0.31 | 0.16 | **0.08** |
| | | | AC | **0.08** | 0.16 | 0.31 | **0.04** | 0.63 |
| *Vangueria infausta* Burch. | Rubiaceae | UNIN 121016 | WA | 1.25 | 0.63 | >2.5 | **0.04** | 0.16 |
| | | | PE | 0.63 | 0.31 | 0.63 | 0.63 | 0.31 |
| | | | EA | 0.31 | 0.16 | **0.08** | 0.31 | 0.31 |
| | | | AC | 0.16 | 0.16 | 0.16 | **0.08** | 0.31 |
| Amphotericin B (µg/ml) | | | | 187.50 | 11.72 | 23.44 | 23.44 | 93.75 |

Values highlighted in bold indicate very strong antifungal activity with MIC value less than 0.1 mg/ml.
WA : Water, PE : Petroleum ether, EA : Ethyl acetate and AC : Acetone.

*Fusarium* species. Water is readily available; therefore, smallholder farmers can prepare crude plant extracts themselves and apply on their fields, gardens or plots to manage *Fusarium* diseases. The use of water extract is also applicable for organic farming. Furthermore, all the extracts were prepared from the leaves of these species. The use of leaves is sustainable from a conservation point of view as leaves are a renewable part that can be safely harvested without threatening plant growth and survival.

Of the 11 medicinal plants investigated, *Combretum erythrophyllum* acetone extract was particularly the most active as it demonstrated very strong activity (MIC<0.1 mg/ml) against four pathogens (*F. equisite, F. oxysporum, F. chlamydosporum* and *F. subglutinans*) and a strong activity (MIC<1 mg/ml) against *F. semitectum.* The antimicrobial activity of isolated compounds such as apigenin, genkwanin and 5-hydroxy-7, 4′-dimethoxyflavone from *C. erythrophyllum* was reported (Alexandra *et al.,* 1992; Martini and Eloff, 1998; Martini *et al.,* 2004). Strong antifungal activity of *C. erythrophyllum* extracts reported in the current study may be due to the presence of these compounds and other flavonoids. Compared to all the medicinal plants evaluated, *Harpephyllum caffrum* seemed to be less active with only its ethyl acetate extract demonstrating very strong activity against *F. subglutinans.*

Of all the pathogens used in this study, *F. subglutinans* was the most susceptible pathogen with 18 extracts exhibiting very strong inhibitory activity against it. On the other hand and in comparison to other pathogens, only three extracts (acetone extracts from *C. erythrophyllum*, *Solanum mauritianum* and ethyl acetate extract from *Quercus acutissima*) demonstrated strong inhibitory activity against *F. equisite*. It was of particular interest that the inhibitory activity demonstrated by the aforementioned extracts was higher than that of the positive control used in this study against *F. equisite*.

Minimum inhibitory dilution (referred to as 'total activity' by some authors) is also an important factor to be considered when evaluating the antifungal activity of medicinal plant extracts, especially if they are intended for practical application. Minimum inhibitory dilution indicates the volume (ml) of solvent which can be added to crude extract obtained from one gram of ground, dried plant material and still inhibits the growth of the pathogen (Eloff, 2004; Amoo *et al.*, 2012). Table 2 presents the minimum inhibitory dilution (MID) of the medicinal plant extracts evaluated against the five pathogenic *Fusarium* species. In most cases, water and acetone extracts gave the highest MID, followed by ethyl acetate extract, while petroleum ether extract gave a low MID. Eighteen out of the 44 extracts evaluated had a MID > 1000 ml/g. These extracts are highlighted in bold font in Table 2. Of this number, 15 extracts with MID > 1000 ml/g are either water or acetone extracts. Both solvents but especially water are accessible and affordable to smallholder farmers to prepare crude extracts for application. The volume of the extract to be prepared will depend on the size of cultivated plot or field and fungal infestation level. In any case, the high MID indicates that small amount of the leaf material will be needed to achieve the desired effect. Overall, *Senna didymobotrya* water extract gave the highest MID against *F. subglutinans* with a recorded value of 6896 ml/g dried powder plant material, followed by *Olea europaea* and *Vangueria infausta* water extracts against *F. chlamydosporum* with recorded MID of 6168 and 5268 ml/g, respectively. The lowest MID was found in petroleum ether extract from *Melia azedarach* against *F. semitectum*. It is noteworthy that the acetone extract of *C. erythrophyllum* which gave a very strong activity (Table 1) against four pathogens (*F. equisite, F. oxysporum, F. chlamydosporum* and *F. subglutinans*) also gave a very high MID (> 1000 ml/g) against the same organisms. Extracts with potent activity (low MIC value) and high MID such as *C. erythrophyllum* acetone extract also had potential to be developed into commercially affordable plant based fungicides.

## CONCLUSION

Many studies on the antimicrobial activity of medicinal plant species have focused mainly on human or animal pathogens, while very few deal with plant pathogens. Availability of ethnobotanical information on plants used to treat human and animal infections as compared to the treatment of agricultural crops might be a reason behind this. Nevertheless,

**Table 2.** Minimum inhibitory dilution of medicinal plant species evaluated against five *Fusarium* pathogens

| Plant species | Extract | Minimum inhibitory dilution (ml/g) | | | | |
|---|---|---|---|---|---|---|
| | | *F. equiseti* | *F. oxysporum* | *F. semitectum* | *F. chlamydosporum* | *F. subglutinans* |
| *Lantana camara* | WA | 128 | 32 | NA | NA | 505 |
| | PE | 20 | 10 | 10 | 80 | 320 |
| | EA | 66 | 66 | 255 | 8 | 510 |
| | AC | 249 | 63 | 996 | 16 | 996 |
| *Senna didymobotrya* | WA | **1724** | 221 | NA | NA | **6896** |
| | PE | 150 | 77 | 10 | 38 | 299 |
| | EA | 116 | 224 | 29 | 29 | 896 |
| | AC | 125 | 242 | 31 | NA | 483 |
| *Combretum erythrophyllum* | WA | 301 | NA | 152 | 301 | 152 |
| | PE | 135 | **1048** | 67 | 67 | **1048** |
| | EA | 645 | 645 | 333 | **2580** | **2580** |
| | AC | **1355** | **2710** | 350 | **2710** | **1355** |
| *Solanum mauritianum* | WA | **1176** | 75 | NA | NA | **2353** |
| | PE | 32 | 32 | 16 | 32 | 61 |
| | EA | 223 | 223 | 14 | 58 | 447 |
| | AC | 69 | 532 | 17 | 69 | 532 |
| *Melia azedarach* | WA | 140 | 35 | NA | NA | **1105** |
| | PE | 27 | 52 | 3 | 27 | 52 |
| | EA | 84 | 169 | 44 | 21 | 84 |
| | AC | 106 | 106 | 27 | 423 | 212 |
| *Harpephyllum caffrum* | WA | 741 | NA | NA | NA | 184 |
| | PE | 117 | 117 | NA | 227 | 117 |
| | EA | 236 | 236 | NA | 30 | 472 |
| | AC | 353 | 182 | 45 | 353 | 182 |
| *Withania somnifera* | WA | 207 | 207 | NA | NA | **1619** |
| | PE | 23 | 89 | 23 | 23 | 179 |
| | EA | 190 | 380 | 760 | 24 | 48 |
| | AC | 153 | 593 | 593 | 75 | 153 |
| *Combretum molle* | WA | 233 | 117 | 59 | **3663** | 117 |
| | PE | 33 | 33 | 16 | 16 | 16 |
| | EA | 177 | 177 | 709 | 709 | 177 |
| | AC | 149 | 288 | 576 | **1152** | 73 |
| *Olea europaea* | WA | 392 | 197 | NA | **6168** | 796 |
| | PE | 22 | 43 | 11 | 675 | 87 |
| | EA | 288 | 288 | 71 | 71 | 288 |
| | AC | 397 | 397 | 98 | NA | 397 |
| *Quercus acutissima* | WA | 121 | NA | NA | 944 | 121 |
| | PE | 32 | 63 | 16 | 252 | 63 |
| | EA | 117 | 235 | 61 | 117 | 235 |
| | AC | 370 | 185 | 96 | 741 | 47 |
| *Vangueria infausta* | WA | 169 | 334 | NA | **5268** | **1317** |
| | PE | 17 | 35 | 17 | 17 | 35 |
| | EA | 54 | 104 | 208 | 54 | 54 |
| | AC | 102 | 102 | 102 | 205 | 53 |

Values highlighted in bold show remarkable minimum inhibitory dilution value of more than 1000 ml/g of dried plant material.

WA : Water, PE : Petroleum ether, EA : Ethyl acetate and AC : Acetone. NA : Not applicable since minimum inhibitory concentration was >2.5 mg/ml.

in the current study, 40, 33, 20, 30 and 40 extracts (which equate to 91, 75, 45, 68 and 91% of the extracts, respectively) demonstrated strong activity (with at least MIC < 1 mg/ml) against *F. equisite, F. oxysporum, F. semitectum, F. chlamydosporum* and *F. subglutinans*, respectively. Some of these extracts manifested antifungal activity that is higher than that of the positive control used in this study. The results of the current study demonstrate the

ability of medicinal plant extracts as alternative agents to fight *Fusarium* pathogens in agricultural sector. The use of renewable part of medicinal plants is affordable, accessible, sustainable and eco-friendly. It also lends itself as a cost-effective option for smallholder farmers. Efforts are underway to establish the *in vivo* antifungal activity of the highly promising medicinal plant extracts and isolate their active compounds.

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